From Page No. 15

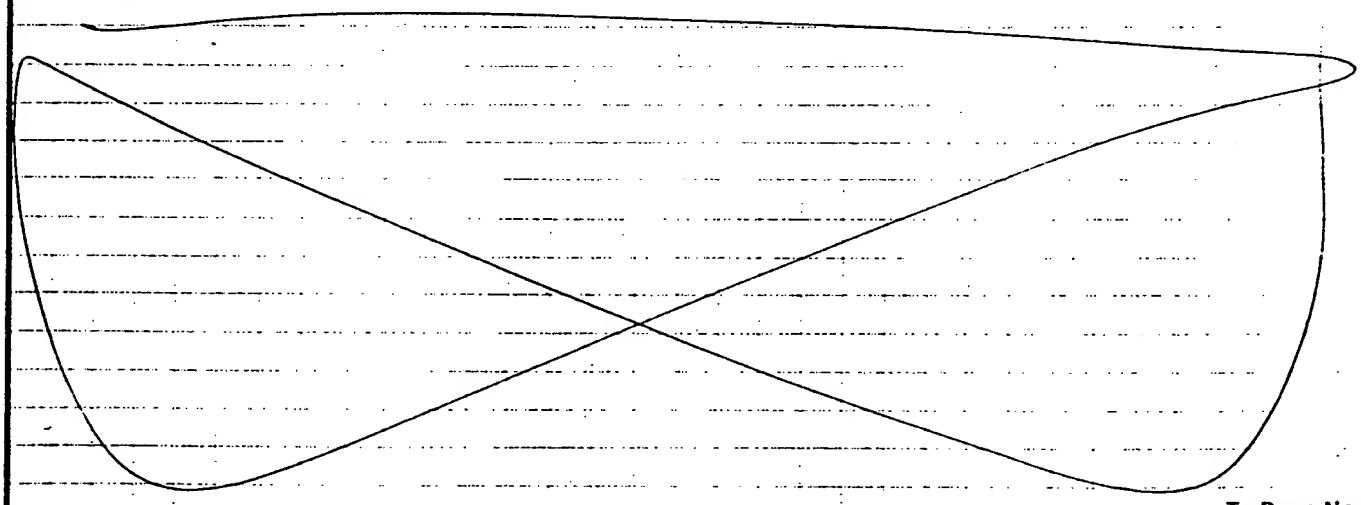
Want to try a serum free (PS04) run on CHO cells to check for Fus expression levels

Have 3 sets of plates currently plus 6 well dishes

Replaced media on 6 well dishes w/ PS04 → Inc

FOR CHO → needs Insulin (bovine)
transferrin
Lipid
trace elements
HEPES

Inc 37°C →



To Page No. 17

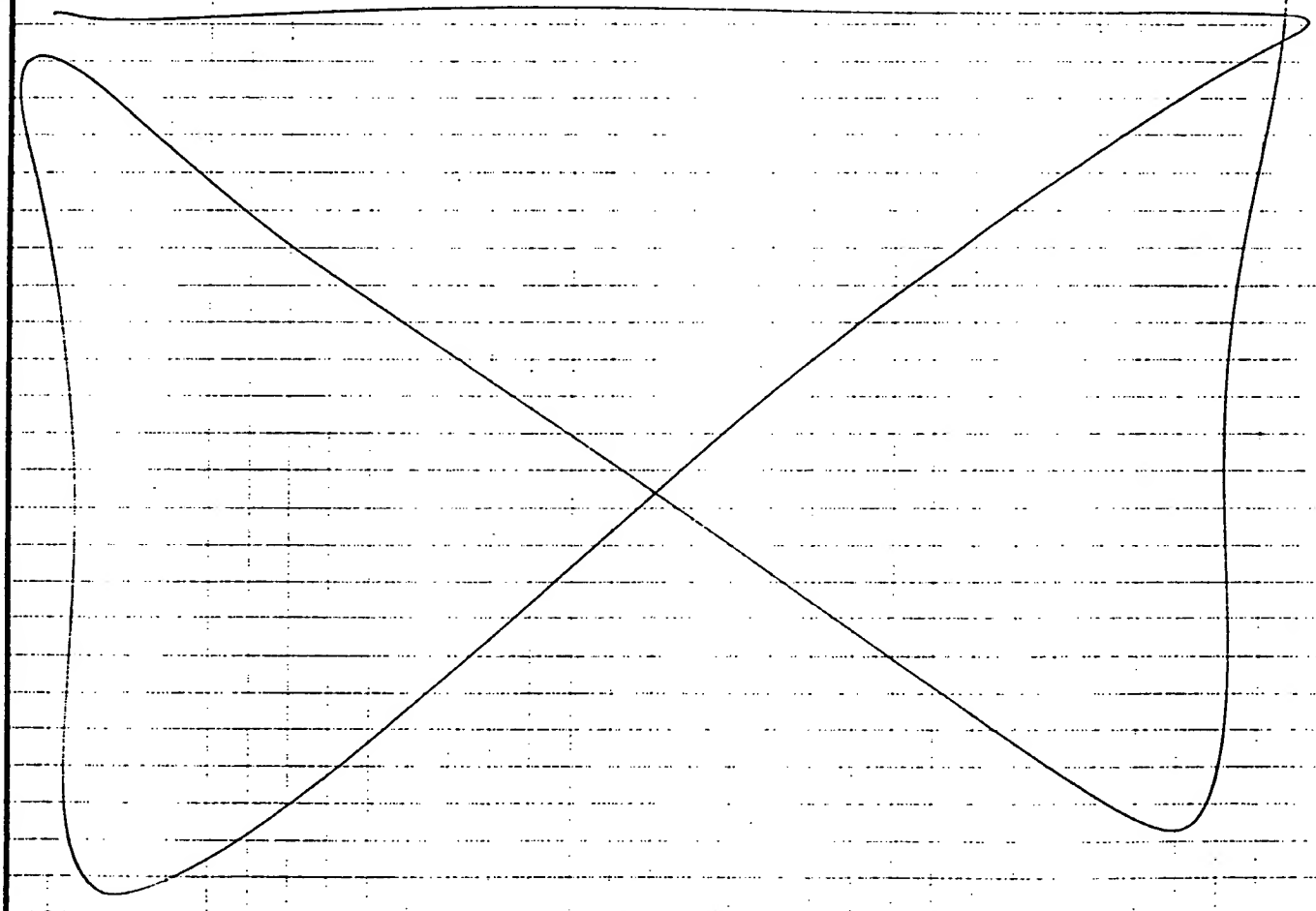
Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		Will Barron	THURS 6/17/93

From Page No. 16

Freeze down 1 set of FHS picks in serum + 7% DMSO -70°C.

Split 10cm plates each 1:10.

Cont PSO4 on 6 well dishes



To Page No. 18

Witnessed & Understood by me,	Date	Invented by	Date FRI
		Recorded by Wm Barron	6/18/93

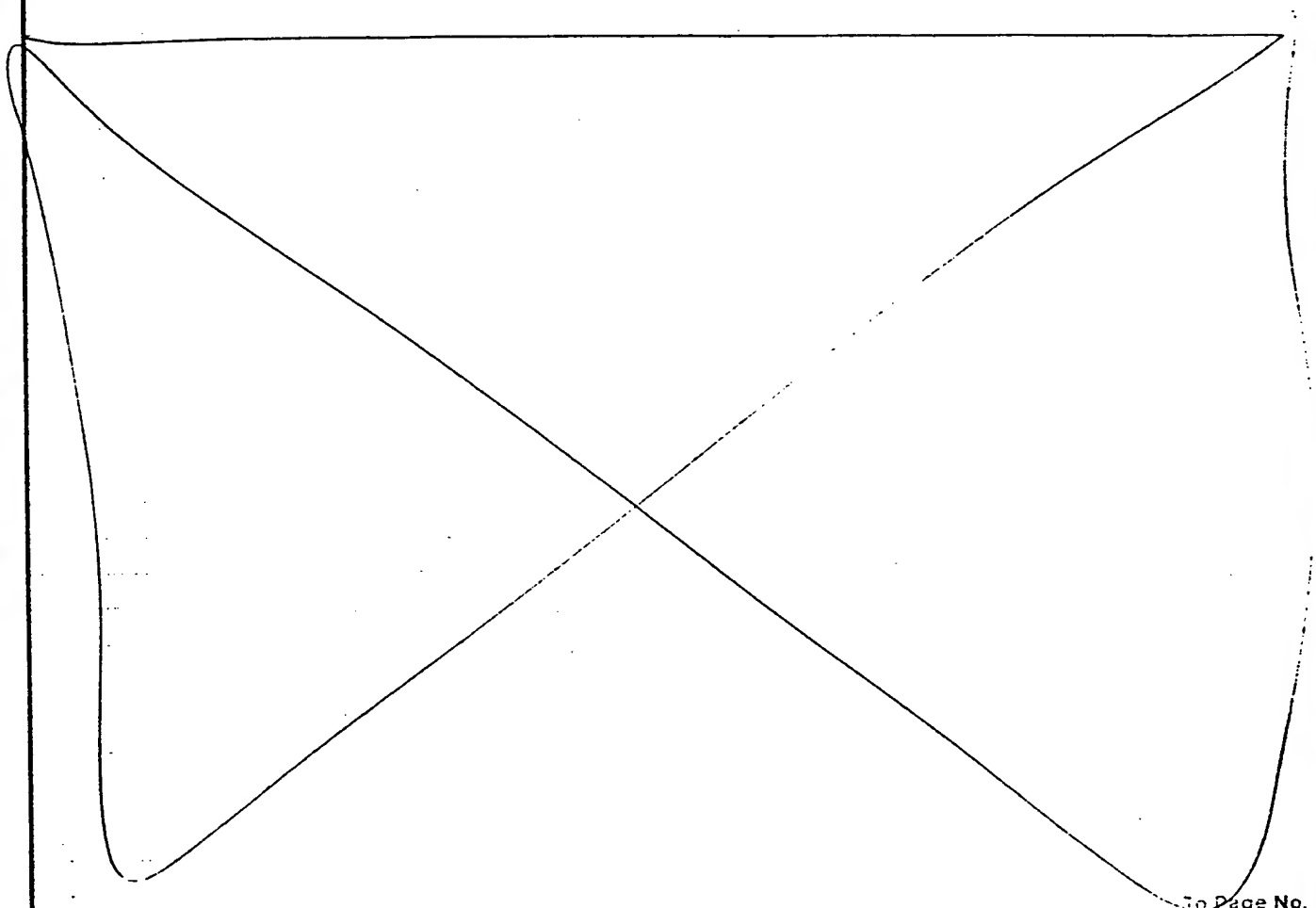
Project No. 1713
Book No. 18802 TITLE

18

From Page No. 17

Harvested PSOY media's
Filtered each Through syringe 0.45μM
Stored -20°C O/N

Split all 36 Fus picks.



To Page No.

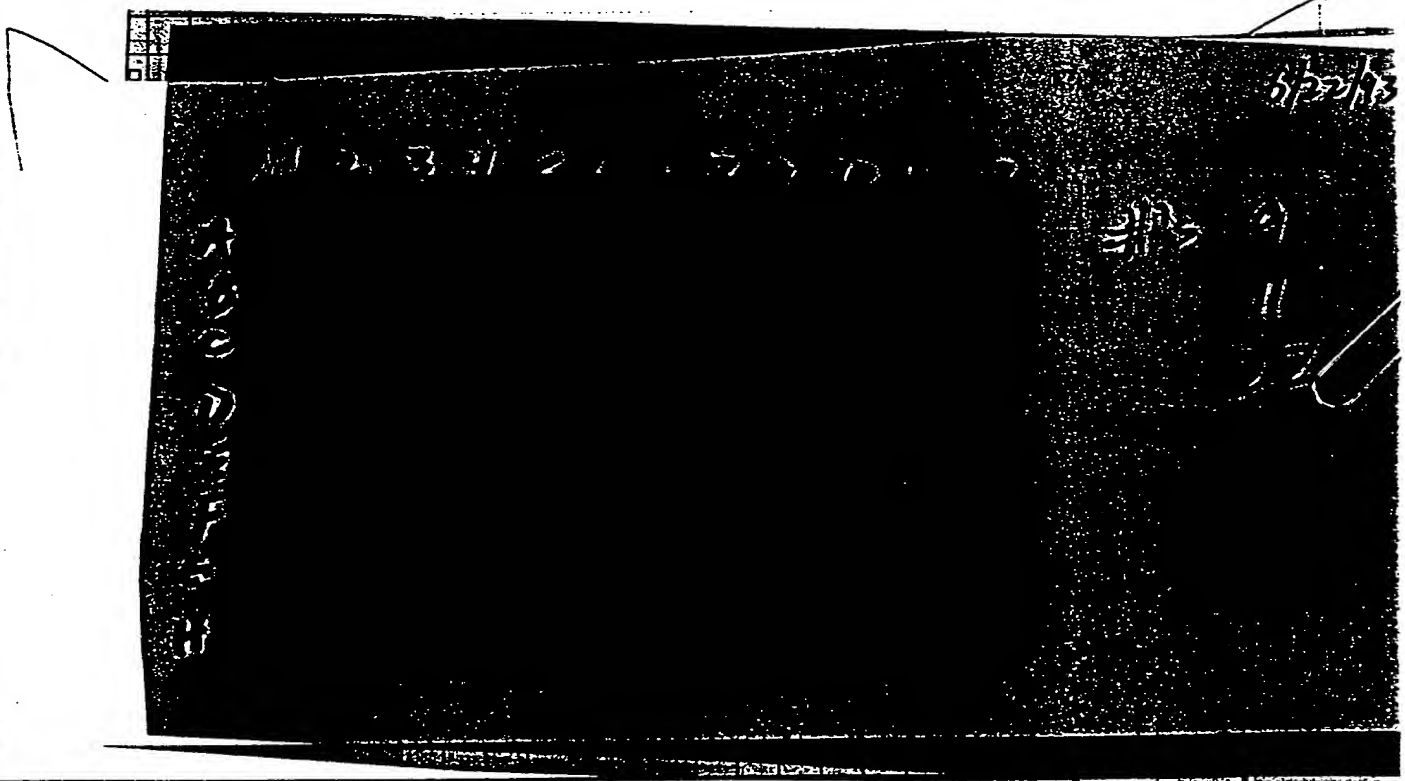
Witnessed & Understood by me,	Date	Invented by	Date MON
		Recorded by Will Bacon	6/21/93

Project No. 1713

TITLE _____ Book No. 18002

From Page No. 12

Dot Blotted 200µl each PSO4 media
Decorated blot w/ 2 Human Fc
ECL Detected
#'s 9, 11 + 22 appear to have highest expression → discarded all cultures except 9, 11 + 22.
Ready to scale-up for Protein production



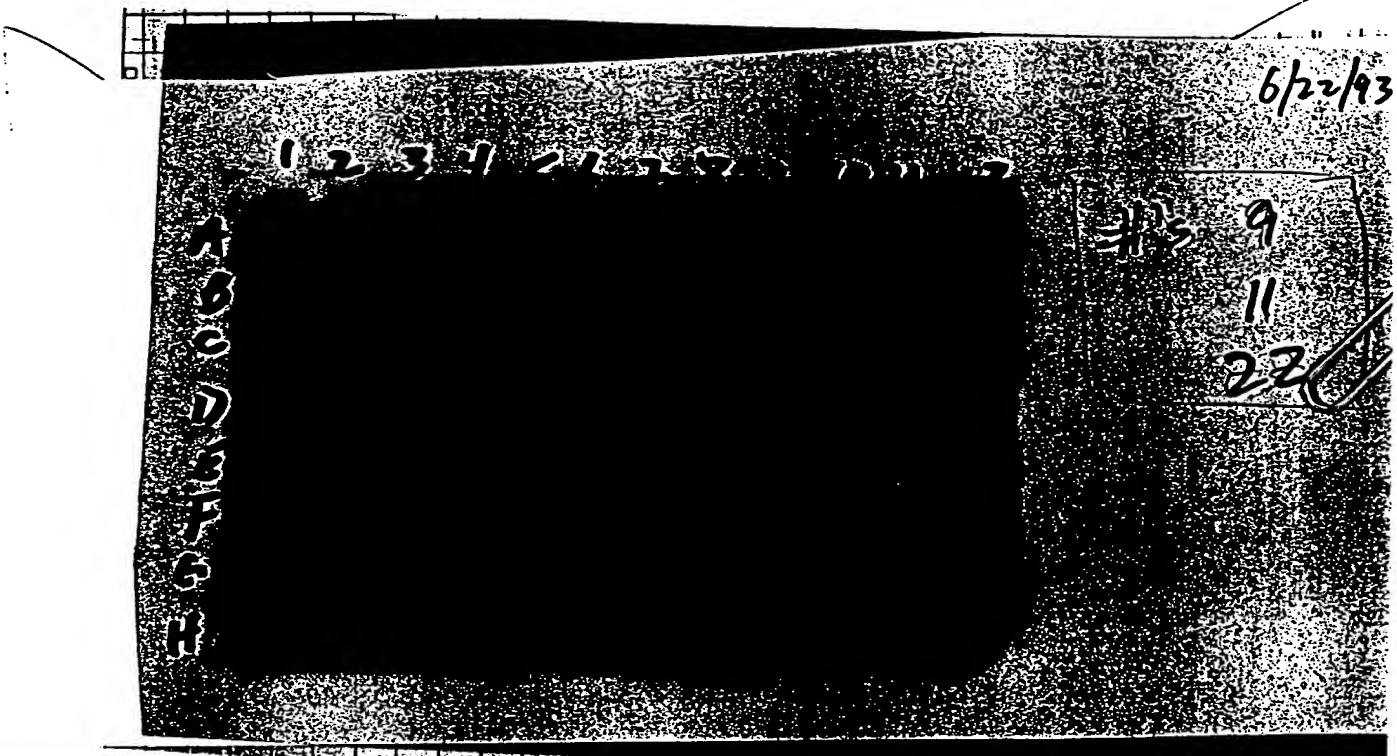
Witnessed & Understood by me.	Date	Invented by Will Bacon	Date TUES 6/22/93
		Recorded by	

9

TITLE

From Page No. 12

Dot Blotted supernatant from media
Decorated blot w/ x Human Fc
ECL Detected
#'s 9, 11 + 22 appear to have highest expression → discarded all cultures except 9, 11 + 22.
Ready to scale-up for protein production



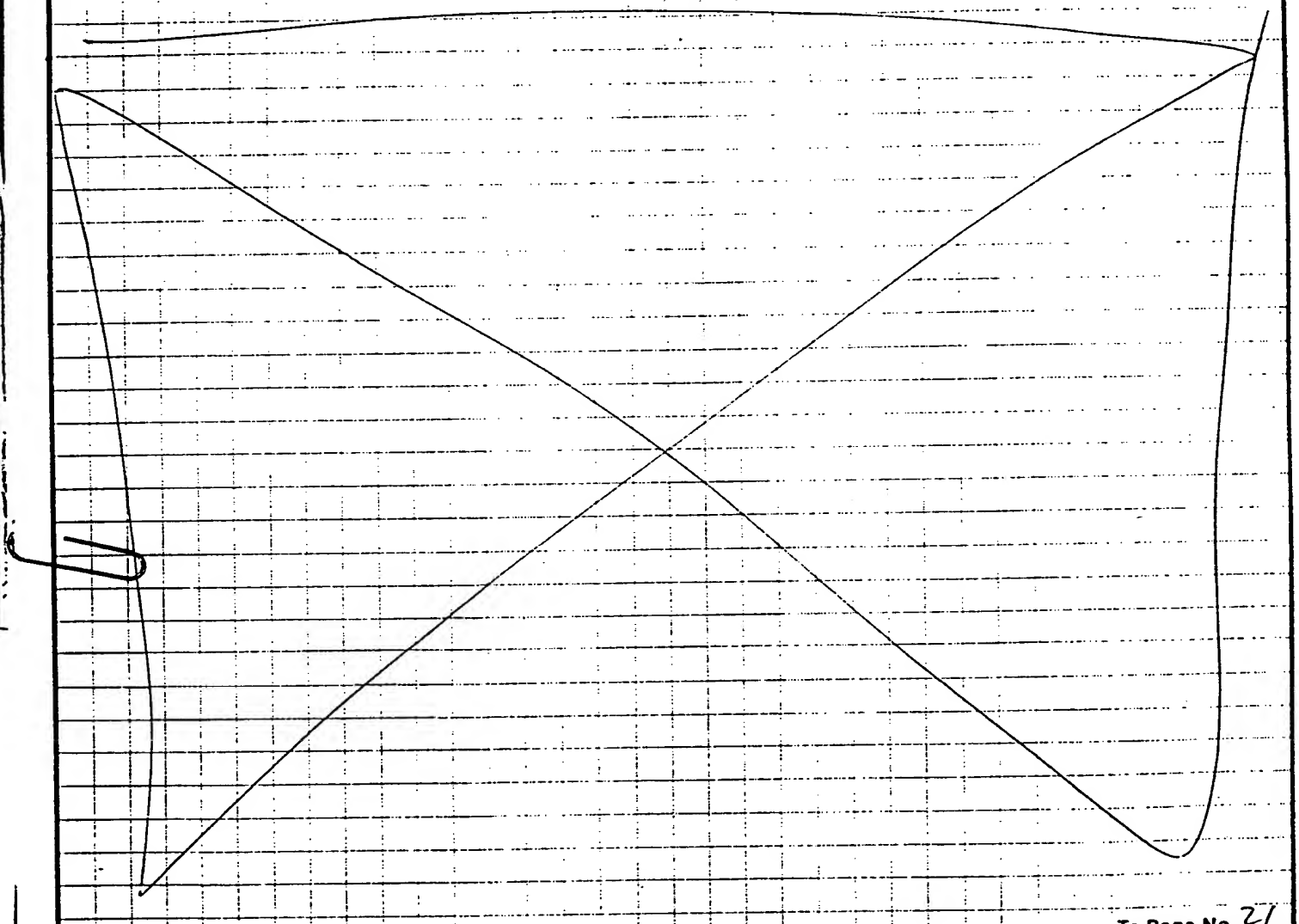
19

Witnessed & Understood by me.	Date	Invented by Will Bowen	Date TUES 6/22/93
		Recorded by	

Project No. 1713
20 Book No. 18002 TITLE

From Page No. 19

Split all FHS samples 1:10 in 10cm dishes (9, 11 & 22)
& 1:5 on 15cm dishes (~~#5~~ #9 only)



To Page No. 21

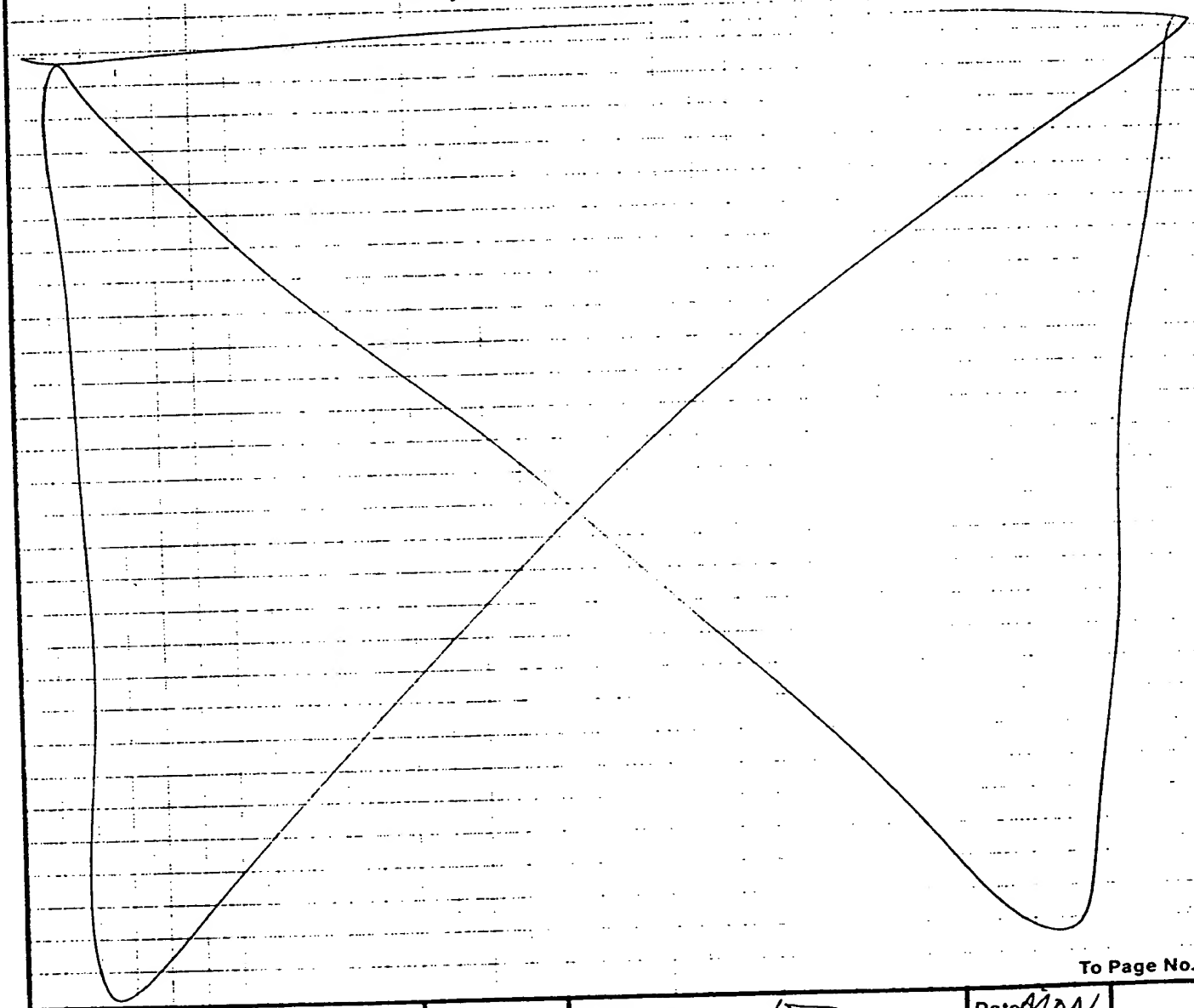
Witnessed & Understood by me,	Date	Invented by	Date FRI 6/25/93
		Recorded by WMBacon	

Project No. 1713
Book No. 18002

TITLE

From Page No. 20

Split Fns 11 & 22 each 1:10 on 10 cm dishes.
Split Fns 9 15cm plate to 5 × 15cm plates
Getting ready for 1st PSO4 run.



To Page No. 2-

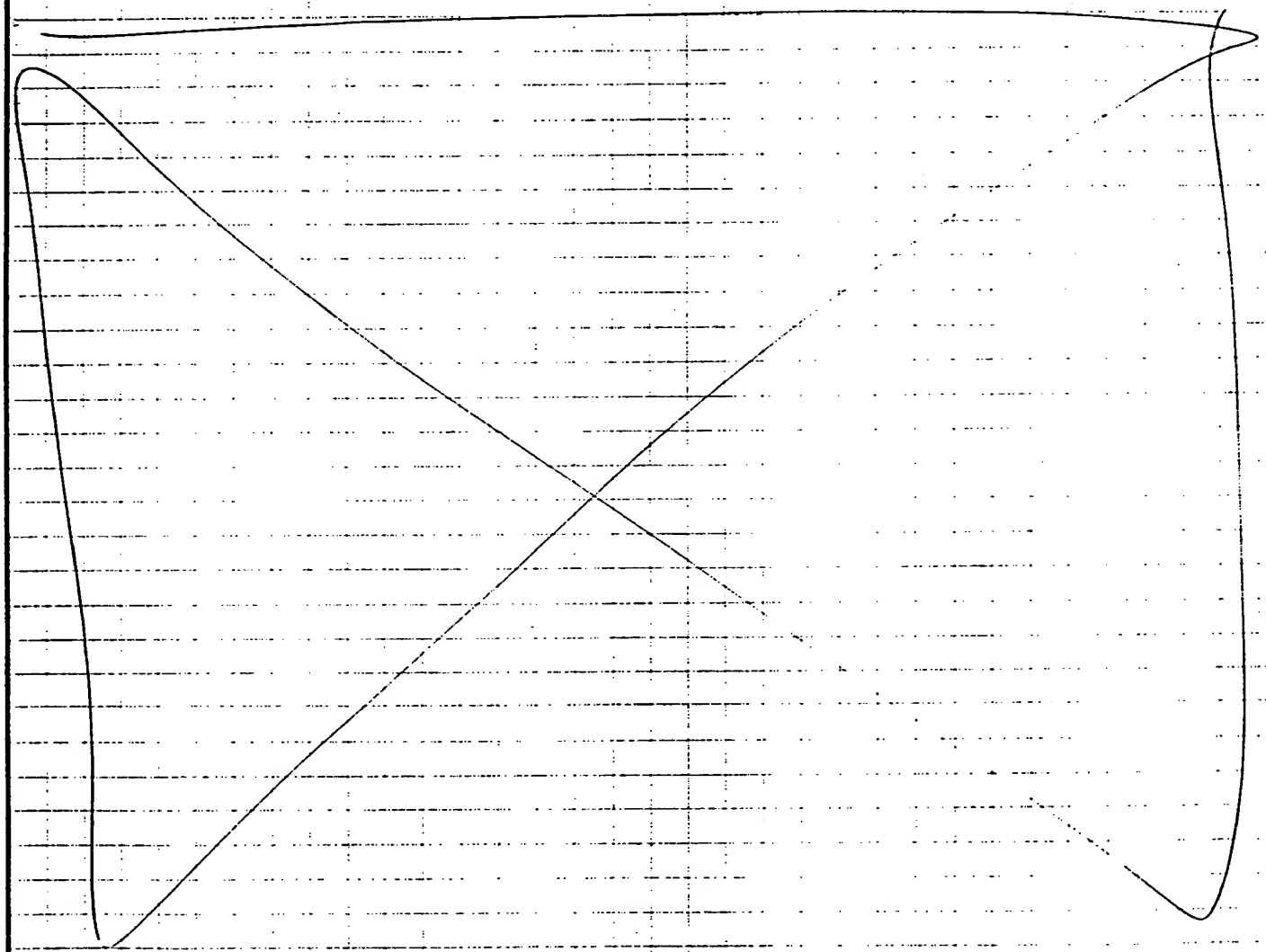
Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		WM Bacon	MON 6/28/93

22 Project No. 1713 U
Book No. 18002 TITLE

From Page No. 21

Split each FUS 9 15cm plate to 8 x 15 cm plates
(= 5 x 8 = 40 total)

Inc o/N 37°C 5% CO_2



To Page No. 23

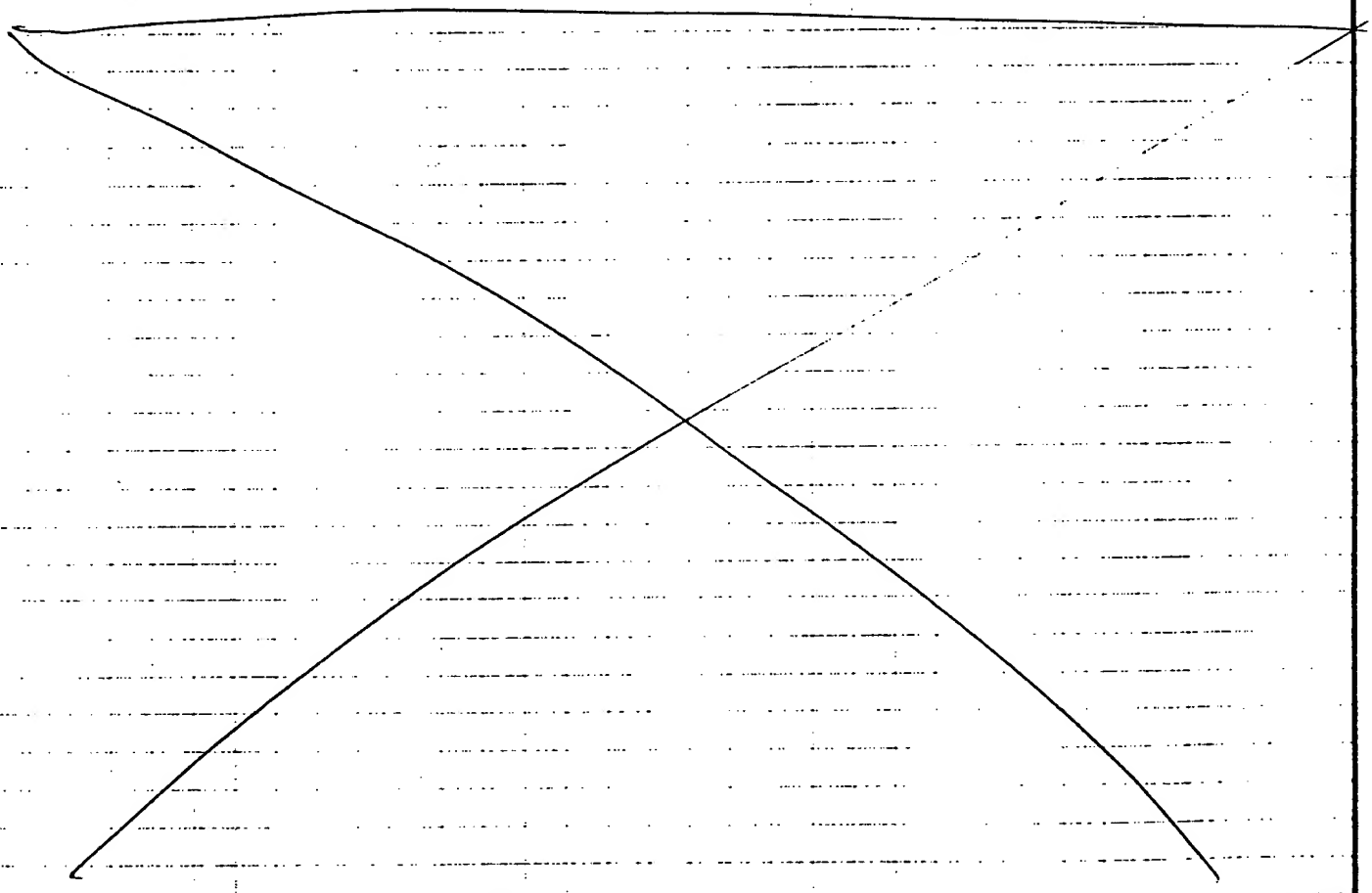
Witnessed & Understood by me,	Date	Invented by	Date WED
		Recorded by Will Bacon	6/30/93

From Page No 22

Changed media on 40 × 15 cm FUS 9 plates to PSO4 → Inc 37°C

Will harvest in ~ 4-6 days (when cells lift off)

Started FUS 9 in 250 ml spinner flask (with 1 entire 16 cm dish worth of cells).



To Page No. 24

Witnessed & Understood by me,	Date	Invented by WM Bacon	Date 5/4/93
		Recorded by	

Project No. 713

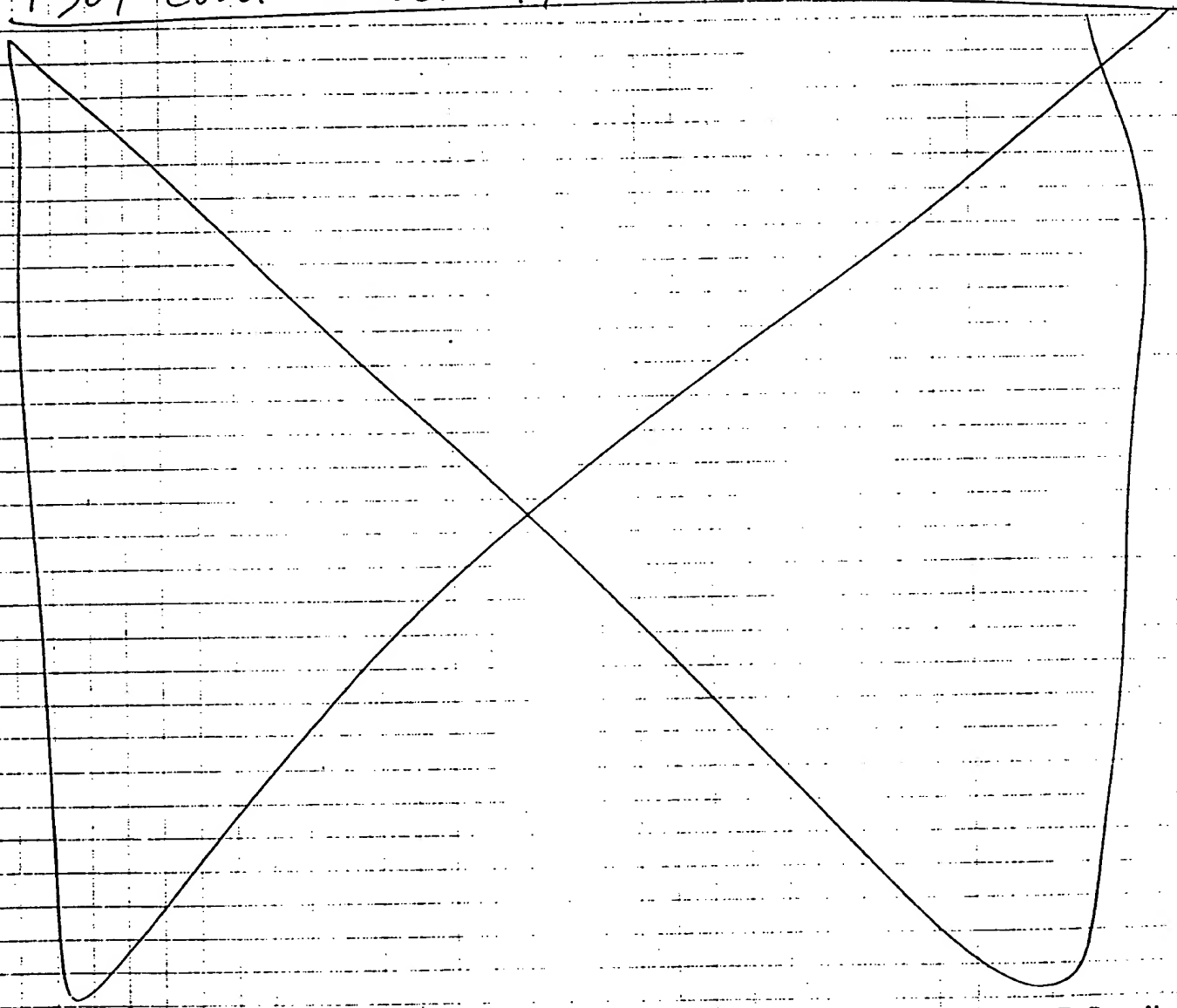
-24 Book No. 18002 TITLE

From Page No. 23

Split Fus 9, 11 + 22 all 1:10

Checked Fus 9 spinners → split 1:10

f504 cont until 7/7



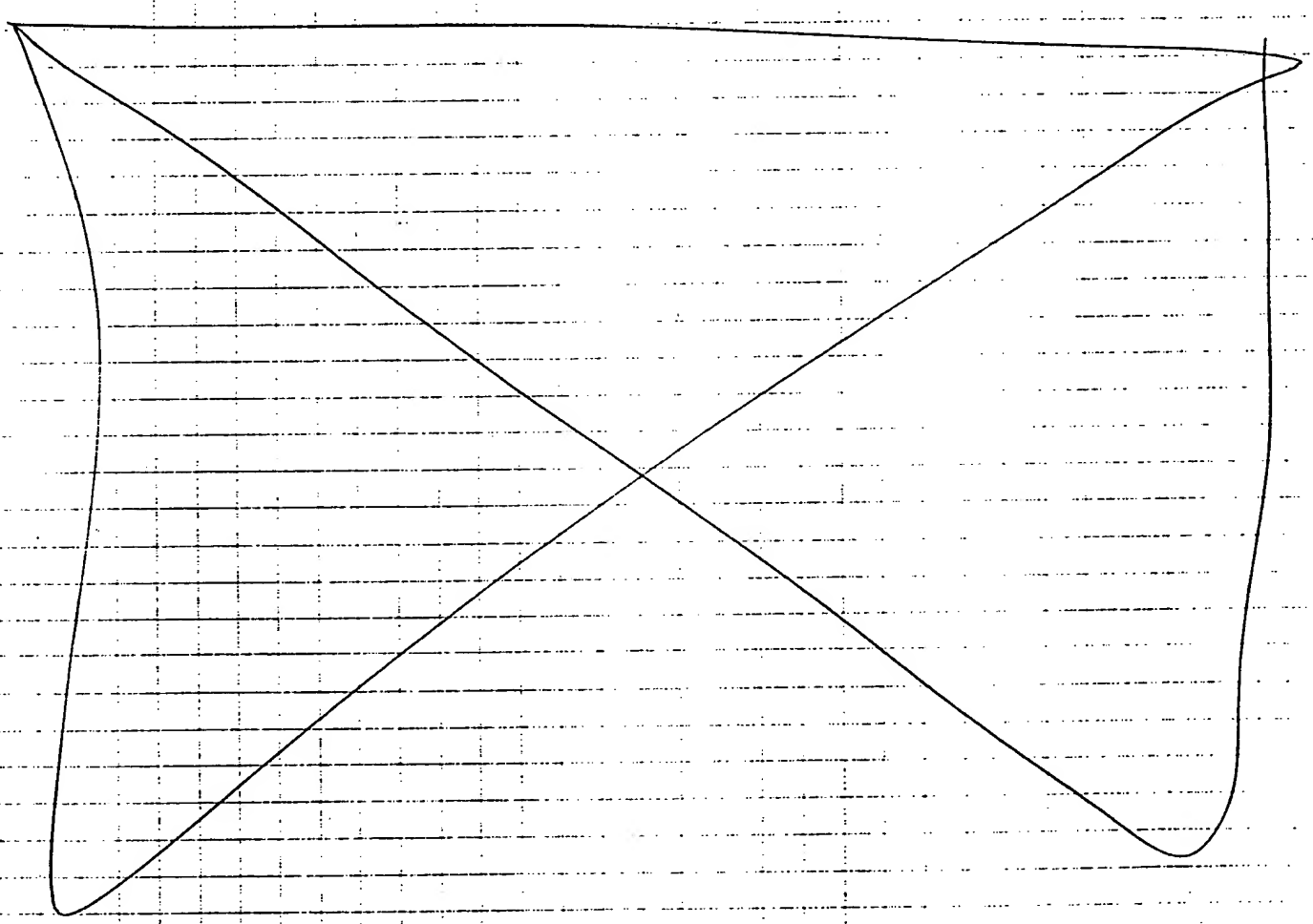
To Page No. 25

Witnessed & Understood by me,	Date	Invented by Will Bacon	Date TUES 7/6/93
		Recorded by	

TITLE

From Page No. 29

Harvested PSO4 media
Filtered Through 0.45μM filter
Added protease inhibitors (PMSF, aprotinin, leupeptin & pepstatin).
Stored 4°C O/N.



To Page No. 26

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		WMP	WED 7/7/93

From Page No. 25

Protein A column procedure:

Column is washed w/ 0.1M Na Citrate pH 6.0

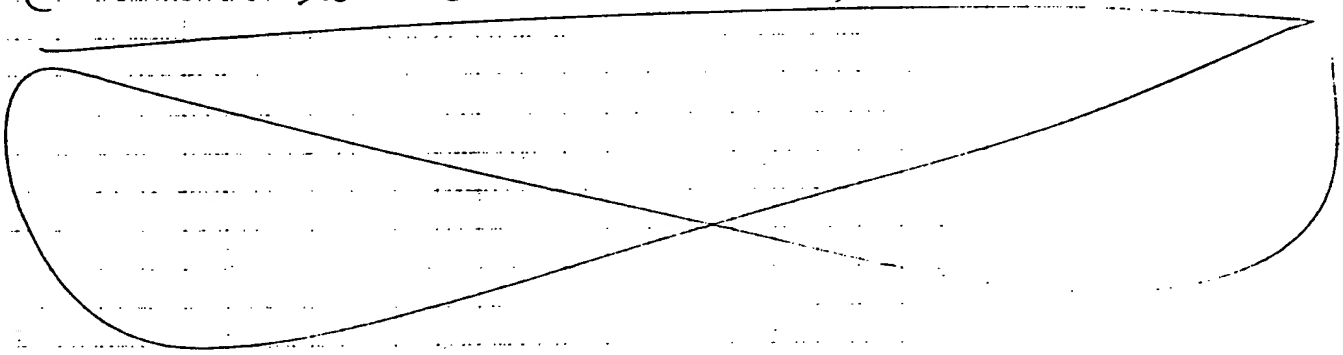
Sample is made up to 0.1M Na Citrate pH 6.0 filtered & then loaded @ ~4ml/min

after loading column is washed w/ citrate

→ when baseline level is reached then sample is eluted w/ 3M $MgCl_2$ elution buffer (see lab protocols)

Sample is desalted on a PD-10 column & concentrated w/ S4 S unit.

Ran entire 1st PS04 batch, washed, eluted, desalted & stored 4°C c/n (Volume is ~3.5ml in PBS)



To Page No. 27

Witnessed & Understood by me,	Date	Invented by Will Bacon	Date THURS 7/8/93
		Recorded by	

TITLE

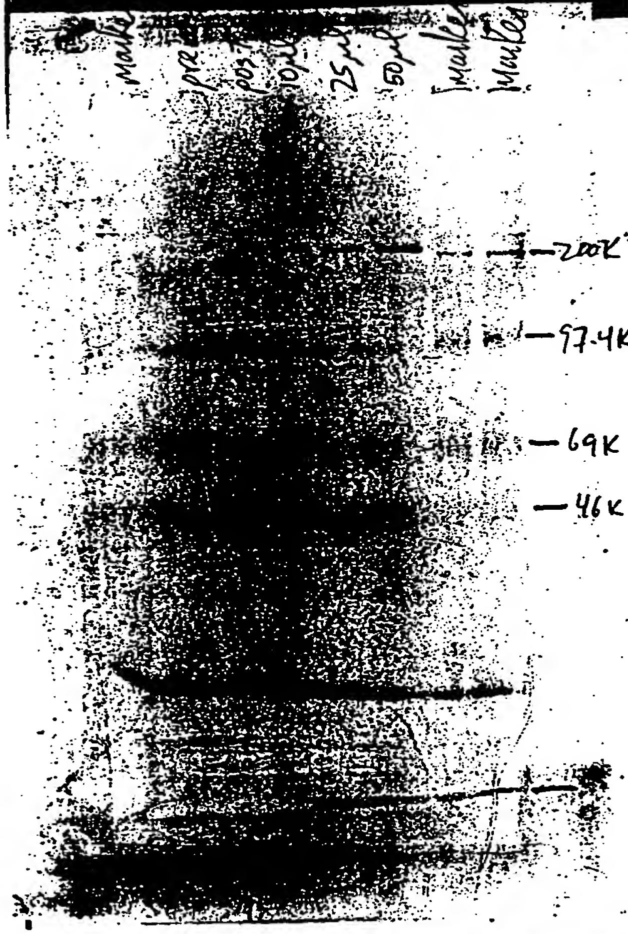
From Page No. 26

Ran samples of Pre Prot A, Post Prot A & of recovered material on 10% SDS-PAGE in duplicate for analysis

MW	MW	50µl REC	25µl REC	10µl REC	5µl POST	50µl PRE	MW	MW	MW	50µl REC	25µl REC	10µl REC	50µl POST	50µl PRE	MW
16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

Cut gel into 2 halves → WESTERN transferred one half & Ponceau'd → other half coomassie stained

[redacted]



Did BCA (on 100µl of 3.5ml) for protein concentration Determination

A_{562} recovered FUS 9 = 0.192
(see standard curve p. 28)

~~[illegible]~~

$y = 29.27(0.192) - 0.60091$
$y = 5.02\ \mu g$ in 100µl $= 50ng/\mu l$

$= 175\mu g$ total yield!

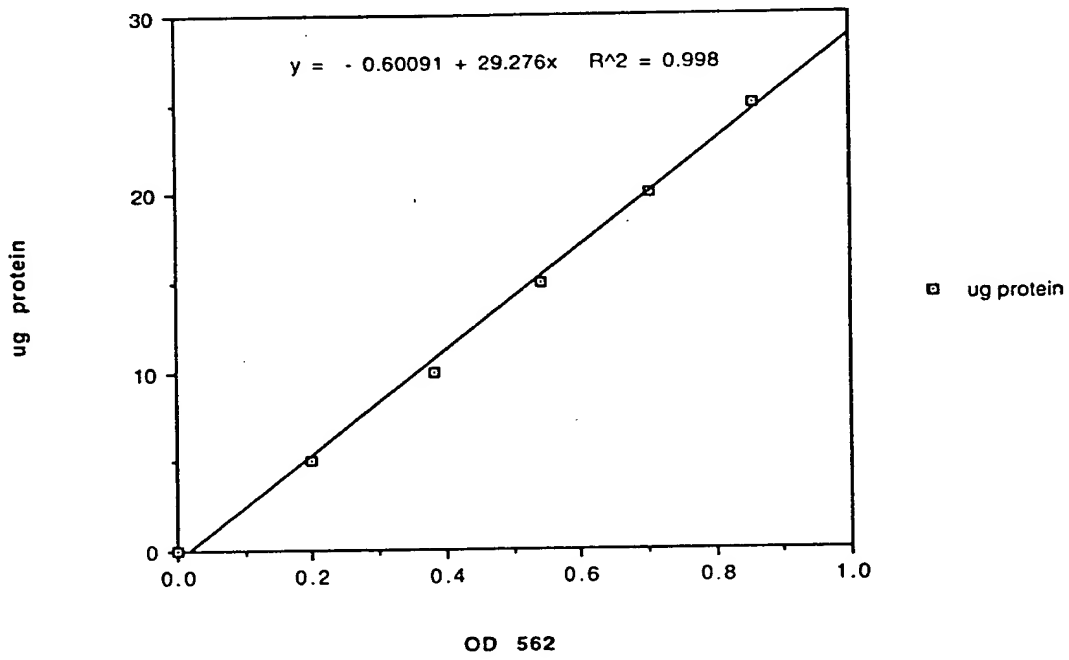
To Page No. 28

Invented by
Recorded by [signature]

Date FRI 7/9/93

17-13
1500 2

27



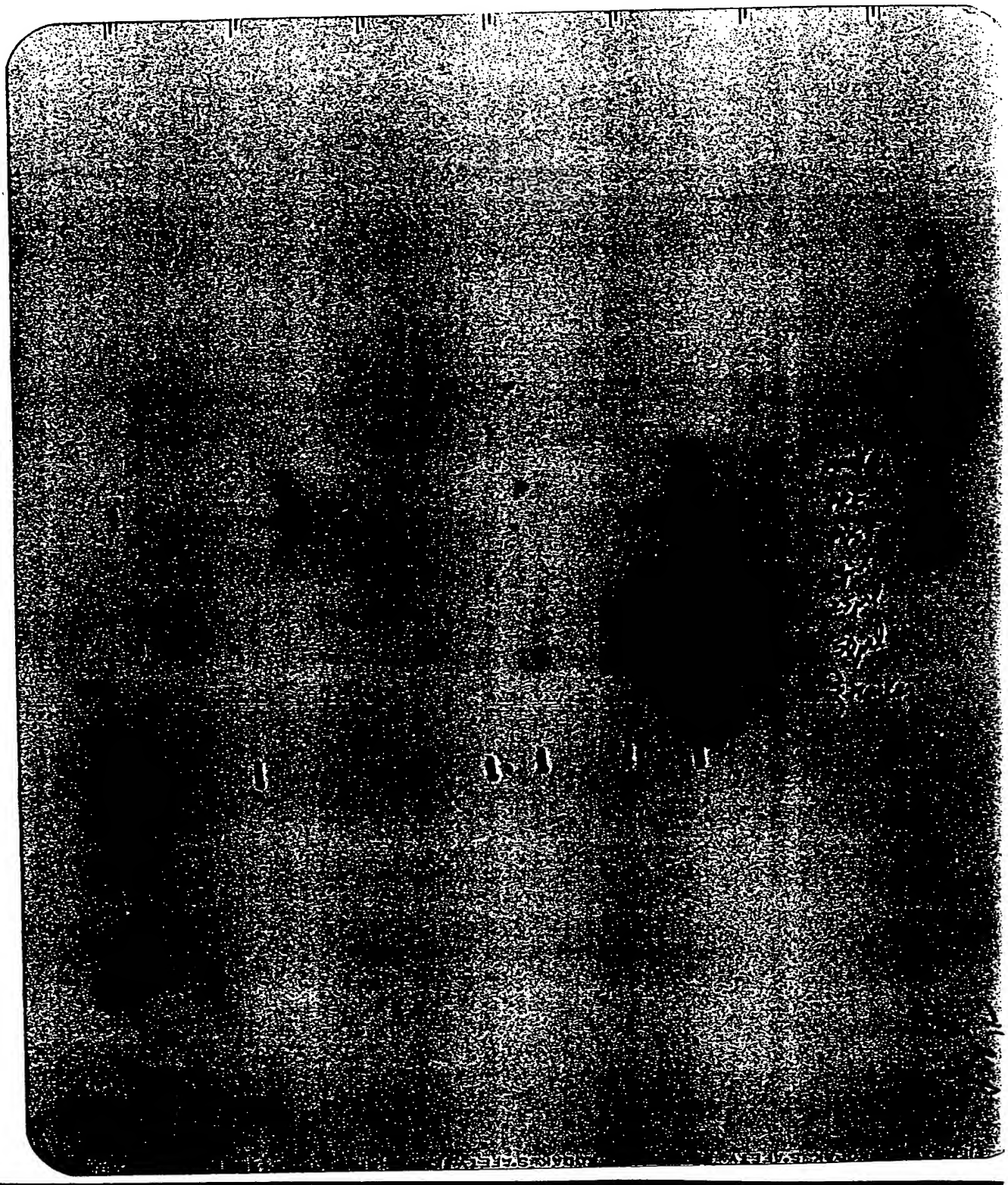
I think that I will get much better yields from spinner culture.

Split Fus 9, 11 & 22 plates all 1:10

Split Fus 9 spinner 1:10

7

Witnessed & Understood by me.	Date	Invented by	Date
		Recorded by HW Bacon	7/9/03



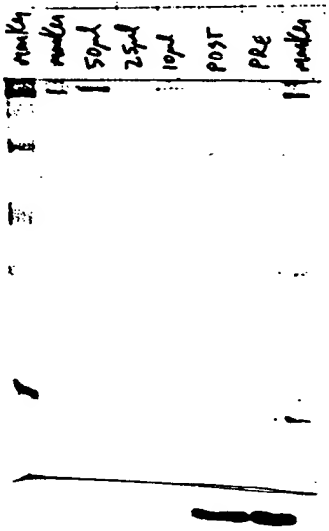
From Page No. 28

Decorated FUS 9 western w/ α Human Fc

ECL detected — Many species observed including some probable degradation produc

Photographed coomasie stained portion of gel

here major material is seen at ≥ 200K



FUS 9

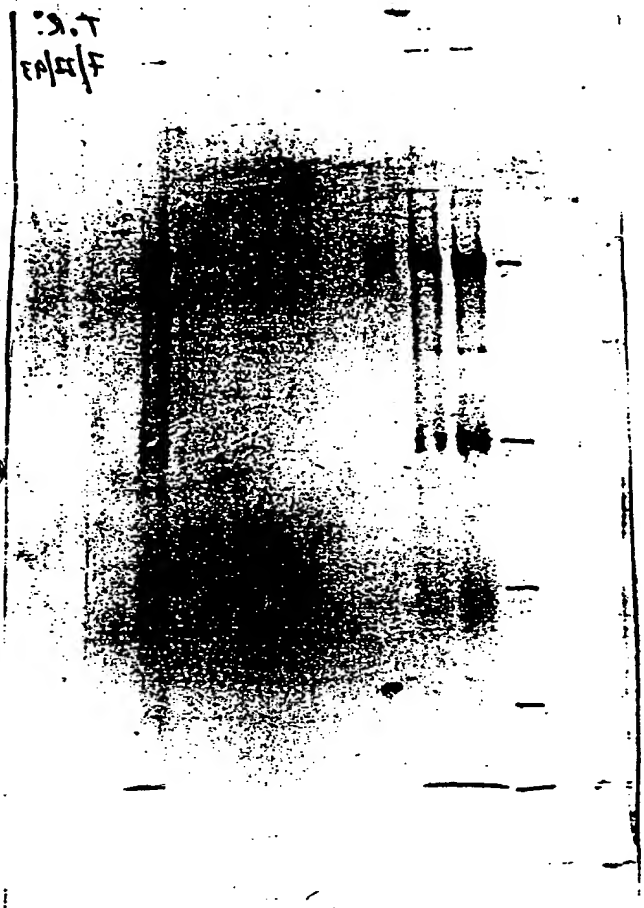
WESTERN!

Split all wells + spinner

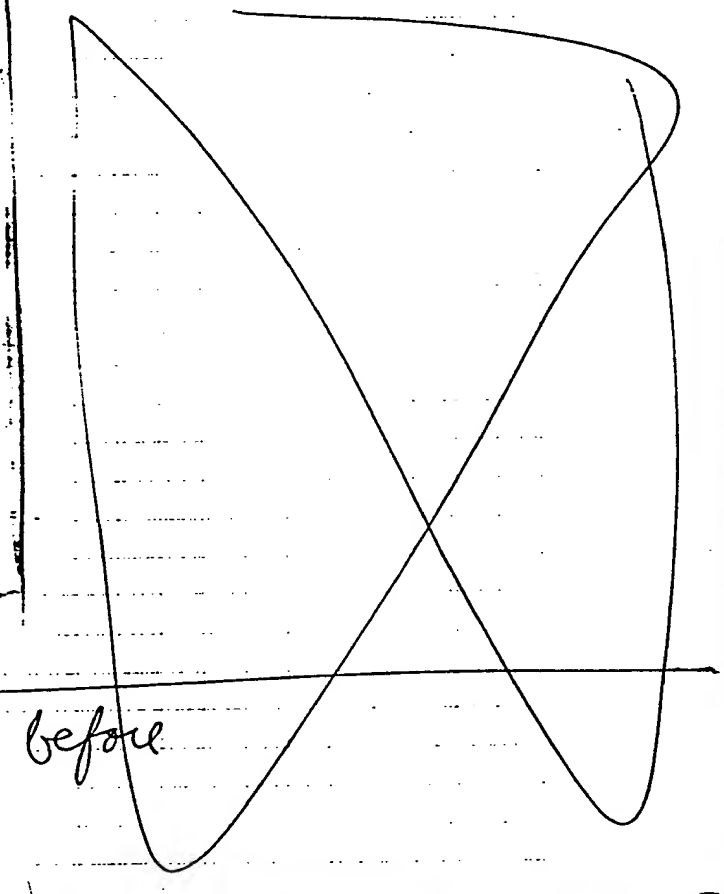
Witnessed & Understood by me,	Date	Invented by WMB	Date MON 7/12/93
		Recorded by WMB	

From Page No. 33

Stopped O/N gel → cut into 2 halves
Coomassie Stained ½ WESTERN transferred ½ Then half → Ponceau stained



Stored blot RT
Destained Coomassie O/N



Started PSO4 run #4 as before

To Page No. 35

Witnessed & Understood by me,	Date	Invented by Wm Barron	Date FRI 7/23/93
		Recorded by	

35

Ed

Page No. 36

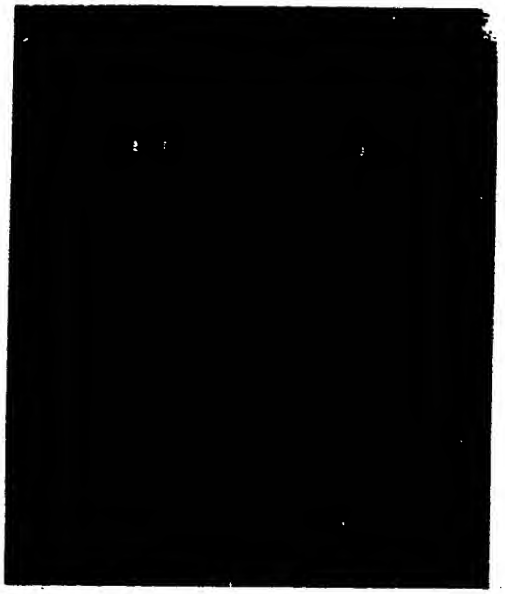
Project No. 1713

TITLE _____ Book No. 18002

From Page No. 34

Decorated Fusion Western w/ α human Fc → ECL detected

Photographed Coomassie stain



FMS

IgG

ECL

To Page No. 36

Witnessed & Understood by me,	Date	Invented by	Date MON
		Recorded by WMBarron	7/26/93

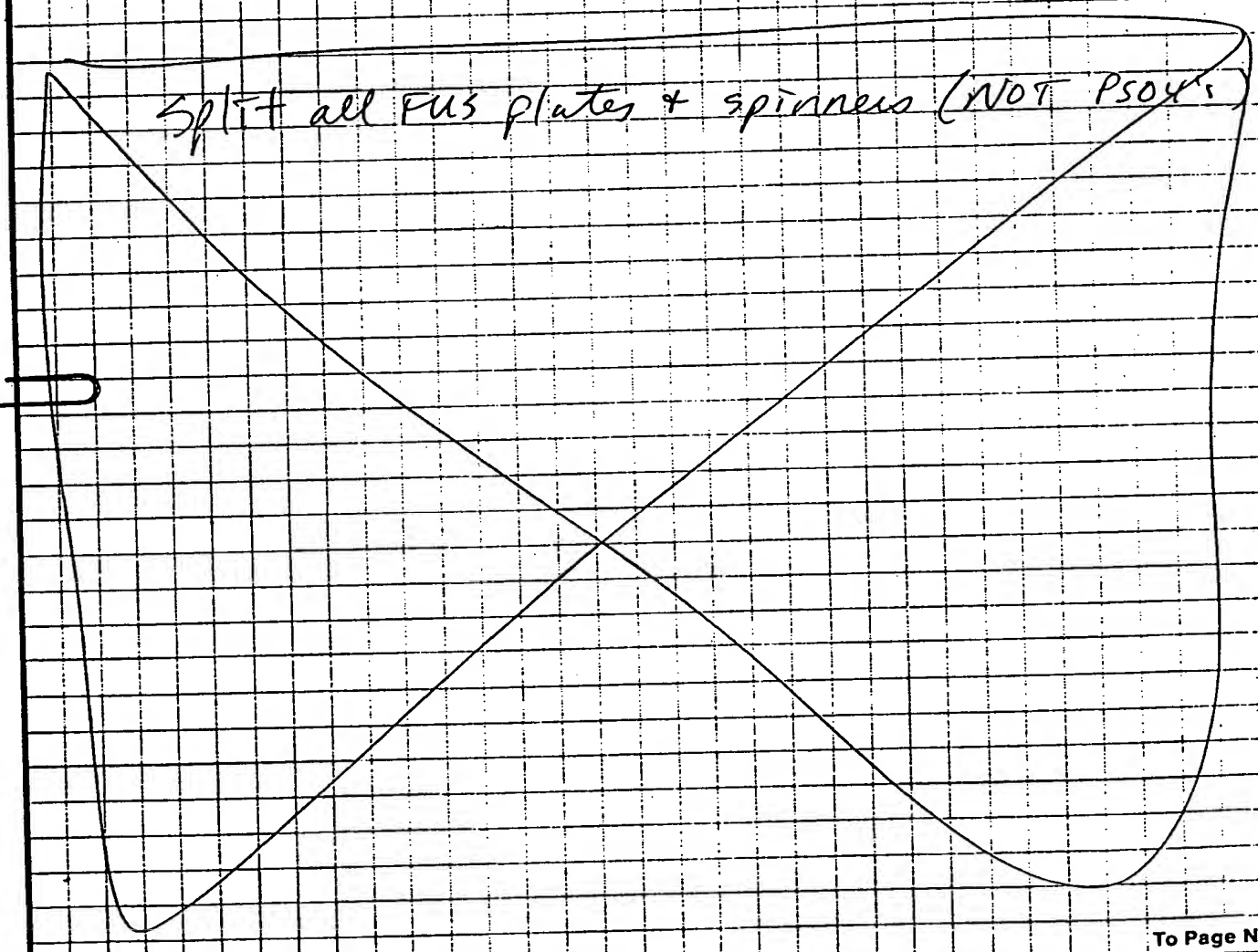
36

Project No. 1713
Book No. 18002 TITLE

From Page No. 35

Harvested 1st PS04 run
Run prot A column
Eluted w/ $MgCl_2$
Desalted, stored 4°C

Split all FU3 plates + spinners (NOT PS04's)



To Page No. 37

Witnessed & Understood by me,	Date	Invented by	Date MON
		Recorded by W.M. Bowen	7/26/93